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U.S.A.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

KNUT A. JAEGGI ET AL

Serial No : 315,962
Filed : February 27, 1989

For : Novel substituted alkanediphosphonic acids

**Declaration under Rule 132**

I, Jonathan Richard Green, a citizen of the United Kingdom of Great Britain and Northern Ireland and a permanent resident at Allschwil, Switzerland declare:

That I am a graduate of the University of Liverpool, England, where I was awarded the Bachelor of Science honours degree in biochemistry in July 1969 and the degree of a Doctor of Philosophy in biochemistry in November 1973;

That I joined the Pharmaceutical Division of CIBA-GEIGY AG of Basle, Switzerland in 1983, where I have acquired special expertise in the pharmacology of bone and related fields;

That I am the author or co-author of several scientific papers on pharmacology and related fields which have been published in scientific journals of high standard, such as Lancet, Biochemical Journal, Agents and Actions, Biochimica and Biophysica Acta and European Journal of Paediatrics;

That I am in charge of testing and evaluating the effects on bone and calcium metabolism of anti-resorptive trial preparations;

That under my guidance and personal supervision comparative tests have been carried out in order to determine and compare the anti-resorptive activities of two pairs of test preparations;

That these two pairs of test preparations were:

A. 2-(1-methylimidazol-2-yl)-1-hydroxy-ethane-1,1-diphosphonic acid (**AI**) according to US Patent Application Serial No. 315,962 and

   3-(imidazol-2-yl)-1-hydroxy-propane-1,1-diphosphonic acid (**AII**) according to US Patent No. 4,687,767;

B. 2-(imidazol-1-yl)-1-hydroxy-ethane-1,1-diphosphonic acid (**BI**) according to US Patent Application Serial No. 315,962 and

   3-(imidazol-1-yl)-1-hydroxy-propane-1,1-diphosphonic acid (**BII**) according to to US Patent No. 4,687,767;

That I have selected the thyroparathyroidectomised rat as the pharmacological model most indicative of the type of bone anti-resorptive utility contemplated in US Patent Application Serial No. 278,394;

That the test procedure applied, the results obtained and my conclusion therefrom are hereinafter described fully and truly:

A. Test Procedure

1,25 dihydroxyvitamin $D_3$-induced hypercalcemia in the thyroparathyroidectomized (TPTX) rat in vivo

The TPTX rat assay was basically performed according to the published method of Trechsel et al (1987) J Clin Invest 80: 1679-1686, with some minor modifications. Thyroparathyroidectomy was performed under anaesthesia (Evipan® Natrium, Bayer, Leverkusen, West Germany, 80 mg/kg i.p.) on male rats, 130-150 g body weight, strain Tif:RAIf (SPF). After a 4-day recovery period the animals were fasted for 24 hours and the serum calcium concentration was determined in a 800 ul blood sample collected from the retro-orbital venous plexus under anaesthesia with oxygen/carbon dioxide (1:1). The serum was diluted 1:30 with $LaCl_3$ (0.1 molar in 0.6 molar HCl) and the calcium concentration was determined by atomic absorption spectrophotometry. The thyroparathyroidectomy was considered to be complete if this control calcium value was below 1.88 mmol/l; animals with higher values were discarded. The remaining animals were divided into groups of 5.

On post-operative days 5 to 8, each group of rats was given a daily injection of 1,25 dihydroxyvitamin $D_3$ (125 pmol/kg s.c., supplied by Hoffmann-La Roche, Basle, Switzerland) together with various doses of the test compound either subcutaneously or orally. After a 24-hour fasting period an orbital blood sample was collected under anaesthesia on day 9 and the serum calcium concentration was determined as described above.

In each experiment the upper and lower limits of hypercalcemia were established with control groups of animals receiving only 1,25 dihydroxyvitamin $D_3$ or saline (100 % and 0 % hypercalcemia, respectively). The dose required to inhibit the induced hypercalcaemia by 50 % ($ED_{50}$) was determined graphically.

Solutions of the sodium salts of bisphosphonates were prepared in water followed by dilution to the required concentration with 0.1 % bovine serum albumin in phosphate-buffered saline (BSA/PBS). When only the free bisphosphonic acid was available, this was first dissolved in an equimolar amount of NaOH and then diluted in BSA/PBS.

B. Results

The results obtained are compiled in the following table

| Test Compound | Inhibition of bone resorption TPTX-Vit $D_3$ model (rat) $ED_{50}$ [mg/kg s.c.] |
|---|---|
| AI | 0,0006 |
| AII | 0,035 |
| BI | 0,00005 |
| BII | 0,045 |

C. Conclusions

The results presented hereinbefore clearly show that all four test compounds AI, AII, BI and BII exhibit strong to very strong inhibitory effects on the resorption of calcium from the bone. However, the result also show that within either pair A and B the compound according to US Patent Application Serial No. 315,962 is by a factor of 58 (AI and AII) or 900 (BI and BII), resp. more active than the corresponding compound according to US Patent No. 4,687,767

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statement and the like so made are punishable by a fine or imprisonment or both under § 1001 of Title 18 of the United States Code and such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed at Basle, Switzerland November 22, 1989

(Jonathan R. Green)

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

I, MOIRA ANN DLUGOSZ, B.A., declare

1. That I am a citizen of the United Kingdom of Great Britain and Northern Ireland, residing at The Manor House, Rowde, Devizes, Wiltshire, SN10 2ND.

2. That I am well acquainted with the German and English languages.

3. That the attached is a true translation into the English language of Swiss Patent Application No. 4666/86-0 filed on 21st November 1986.

4. That all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardise the validity of the patent application in the United States of America or any patent issuing thereon.

Declared this 6th day of November 1989.

M.A. DLUGOSZ